Expression of Peroxiredoxin 1, 2, 3, and 6 Genes in Cancer Cells during Drug Resistance Formation

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We studied the expression of peroxiredoxin genes (*PRDX1*, *PRDX2*, *PRDX3*, and *PRDX6*) in human erythroleukemia K652, human breast carcinoma MCF-7, and human ovarian carcinoma SKOV-3 cells during cisplatin resistance development. It was found that drug resistance formation was accompanied by a significant increase in the expression of *PRDX1*, *PRDX2*, *PRDX3*, *PRDX6* genes in all cancer cell strains, which confirms the important contribution of redox-dependent mechanisms into the development of cisplatin resistance of cancer cells.

Key Words: *drug resistance of tumor cells; gene expression; peroxiredoxin; cisplatin*

Peroxiredoxins (Prxs, EC 1.11.1.15) constituting a family of Se-independent peroxidases participate in degradation of H_2O_2, organic hydroperoxides, and peroxynitrite, are expressed in practically all organs and tissues, exhibit certain tissue specificity, and can be divided into 3 subfamilies: two-cysteine (Ptx1-4), atypical two-cysteine (Prx5), and singe-cysteine (Prx6) Prx [1,4,12]. All Prxs have a conserved N-terminal Cys residues and Prxs1-4 also carry C-terminal Cys residues. A certain specificity was revealed in subcellular localization of Prxs: Prx1, Prx2, and Prx6 were detected in the cytoplasm, Prx4 in the endoplasmic reticulum, Prx3 in mitochondria, and Prx5 in peroxisomes, nucleus, and mitochondria [2]. These enzymes exhibit antioxidant properties, regulate cell signaling and proliferation, protect protein structure acting as molecular chaperones, and inhibit redox-dependent pathways of apoptosis activation, which attests to their important role in cell protection from oxidative stress [2,12]. High level of Prx1, Prx2, Prx3, and Prx6 gene expression was detected in some tumors [10,11,13]. However, their role in the development of drug resistance of tumor cells remains little studied.

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Here we studied the expression of Prx1, Prx2, Prx3, and Prx6 genes during the formation of drug resistance of tumor cells to cysplatin (CDDP); the cytostatic effect of this preparation, apart from alkylating mechanism, is associated with activation of generation of reactive oxygen species [9].

MATERIALS AND METHODS

The experiments were carried out on K562 (human erythroleukemia; Institute of Cytology, St. Petersburg), MCF-7 (human breast carcinoma), and SKOV-3 cells (human ovarian carcinoma; All-Russian Research Center of Molecular Diagnostics and Therapy) sensitive (IC_{50} 6.0, 13.0, and 2.7 μM for K562/S, MCF-7/S, SKOV-3, respectively) and resistant to cisplatin (IC_{50} 12.7, 30.0, and 12.0μM for K562/S, MCF-7/S, SKOV-3, respectively). K562 cells were cultured as a suspension in RPMI-1640 medium (Sigma) and MCF-7/S and SKOV-3 cells were cultured as a monolayer in DMEM (Sigma). In all cultures, 10% heat-inactivated FCS (Gibco-BRL), 2 mM L-glutamine, 100 μg/ml penicillin, and 50 μg/ml streptomycin were added; the cells were cultured at 37°C in a humid atmosphere with 5% CO_2. Cell resistance to CDDP was induced by incubation in the presence of gradually increasing con-

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centrations in the culture medium; log-phase cells were used in the experiments.

mRNA content was measured by reverse transcription-PCR (RT-PCR). RNA was isolated using RNAwiz kits (Ambion) according to manufacturer's instruction. Total RNA from each sample (~5 μg) were used as the template for the synthesis of cDNA chain using SuperscriptII reverse transcriptase (Invitrogen). PCR conditions were as follows: 3 min at 94°C; 30-36 cycles: 30 sec at 94°C, 20 sec at 55-64°C, and 30 sec at 72°C. PCR products were separated by electrophoresis in 1.5-2.0% agarose gel followed by densitometry. Analysis of gels was carried out using BioCaptMW software (VilberLourmat). The level of mRNA was evaluated relative to β-actin mRNA. The following primers were used: for Prx1 — 5'-CAGCCCAGCGCTCACTTCTGC-3' (forward), 5'-CAGACCCGAAGCGCACCATTGC-3' (reverse); Prx2 — 5'-CTGGCGAAGGACACCCTTGCCATC-3' (forward), 5'-GGCCACAGCGGTGGTTGATGGCG-3' (reverse); Prx3 — 5'-CTTGGTGTATTTATCCAGGCAAGATGGC-3' (forward), 5'-GGCCTGCTGCATGTGGAAGAACGA-3' (reverse); Prx6 — 5'-CCTCTGGCTCACAGCACCAACTTCTCC-3' (forward), 5'-CCTGTGACAGCTCGTGTGGTGTT-3' (reverse); β-actin — 5'-CCACGAAACTACCTTCAACTCC-3' (forward), 5'-TCGTCATACTCCTGCTTGCTGATCC-3' (reverse).

The data were processed statistically using the Student *t* test.

RESULTS

Expression of *PRDX1* gene was similar in all cell strains (Fig. 1, *b*), while expression of *PRDX2, PRDX3*, and *PRDX6* genes was higher in K562/S cells (Fig. 1, *c*; Fig. 2, *b, c*).

In all three CDDP-resistant cell substrains, an appreciable increase in *PRDX1* and *PRDX2* was noted (Fig. 1, *a-c*). The increase in Prx1 mRNA level was maximum (4-fold) in MCF-7/CDDP cells and somewhat less pronounced in K562/CDDP and SKOV-3/CDDP cells (2- and 3-fold, respectively; Fig. 1, *a*, *b*). A more pronounced rise was noted for Prx2 mRNA: by 3, 7, and 6 times in K562/CDDP, MCF-7/CDDP, and SKOV-3/CDDP cells, respectively, in comparison with sensitive cells (Fig. 1, *a, c*). Prx1 is a dominant Prx isoform in many cell types; it exhibits high antioxidant activity and can be induced by elevated concentrations of 4-hydroxy-2-nonenal during LPO activation [5]. H_2O_2 and organic hydroperoxides can also induce *PRDX2* gene expression [12]. Moreover, overexpression of *PRDX1* and *PRDX2* genes protects cells from H_2O_2-induced apoptosis [7].

In all three CDDP-resistant cell substrains, an increase in the expression of mitochondrial Prx iso-

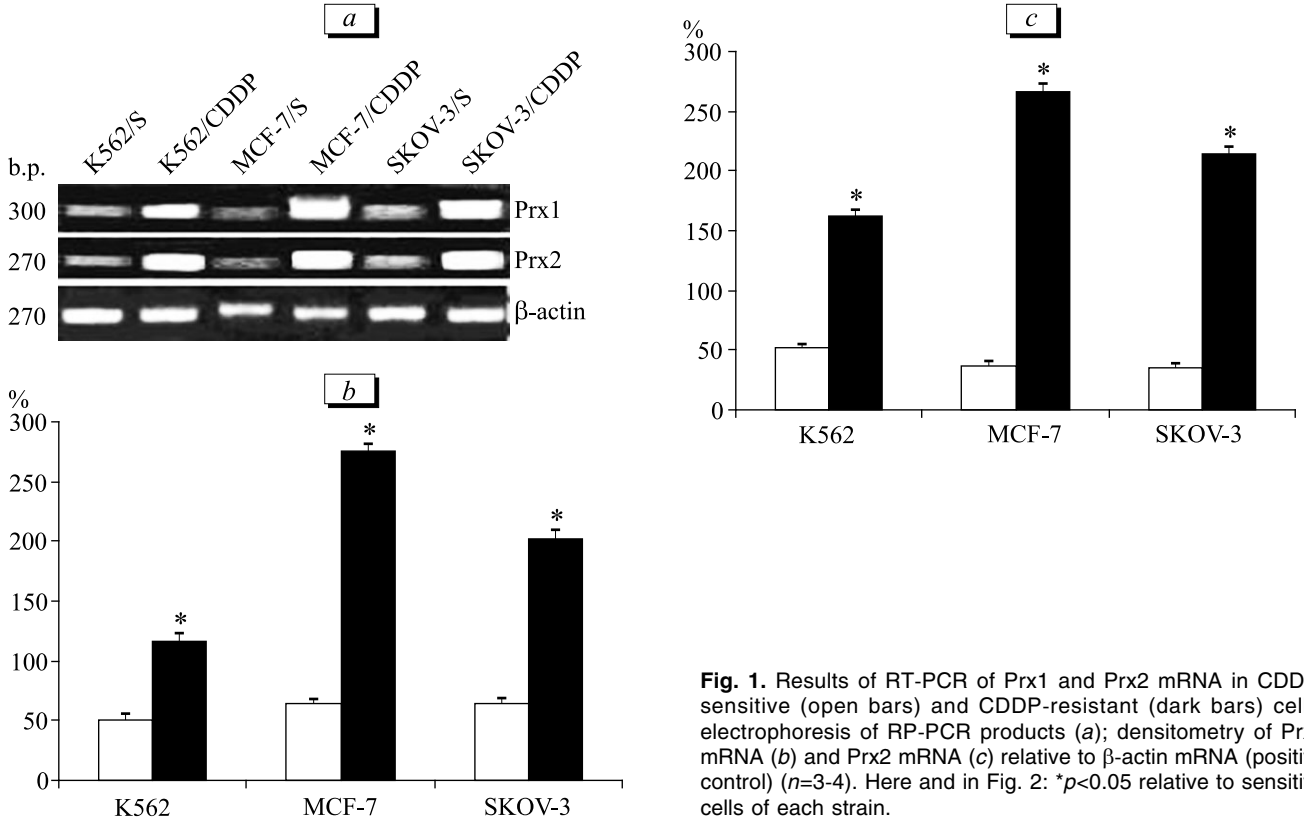

Fig. 1. Results of RT-PCR of Prx1 and Prx2 mRNA in CDDP-sensitive (open bars) and CDDP-resistant (dark bars) cells: electrophoresis of RP-PCR products (*a*); densitometry of Prx1 mRNA (*b*) and Prx2 mRNA (*c*) relative to β-actin mRNA (positive control) (n=3-4). Here and in Fig. 2: *p<0.05 relative to sensitive cells of each strain.

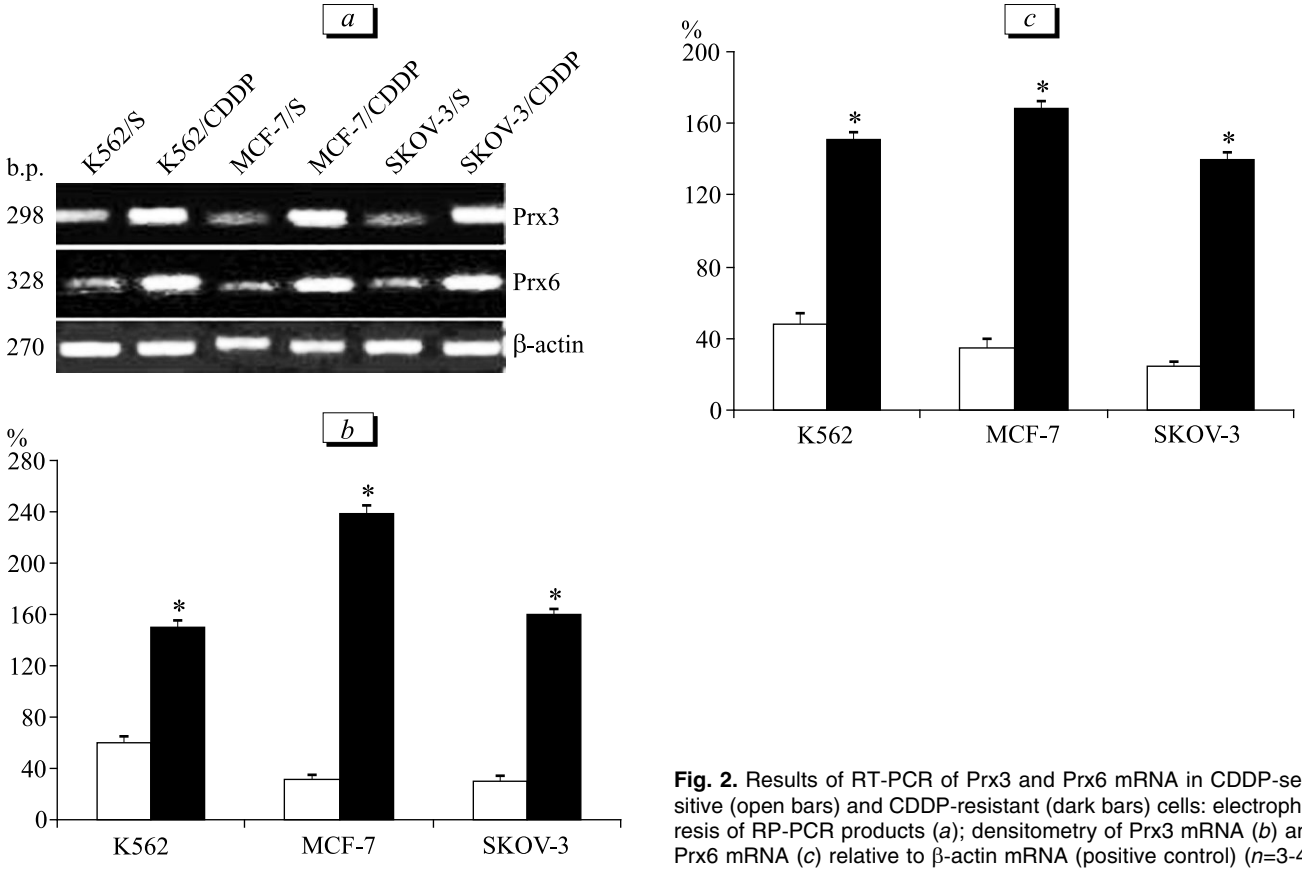

Fig. 2. Results of RT-PCR of Prx3 and Prx6 mRNA in CDDP-sensitive (open bars) and CDDP-resistant (dark bars) cells: electrophoresis of RP-PCR products (*a*); densitometry of Prx3 mRNA (*b*) and Prx6 mRNA (*c*) relative to β-actin mRNA (positive control) (*n*=3-4).

form, Prx3, was observed; this enzyme protects cells from apoptosis and participates in the regulation of cell proliferation [13] (Fig. 2, *a*, *b*). The increase in Prx3 mRNA level was more pronounced in resistant MCF-7/CDDP cells (by 7.7 times) and SKOV-3/CDDP cells (by 5 times) and less pronounced in K562/CDDP cells (by 2.5 times).

A pronounced increase in *PRDX6* gene was also revealed in all three CDDP-resistant cell substrains (Fig. 2, *a, c*). The level of Prx6 mRNA in K562/CDDP, MCF-7/CDDP, and SKOV-3/CDDP cells was higher than in sensitive cells by 3, 4.8, and 5.6 times, respectively.

It should be noted that Prx6, in contrast to other Prx isoforms, uses glutathione (but not thioredoxin) as the source of electrons [2,12]. Moreover, Prx6 protects cells from H_2O_2, and hydroperoxides of fatty acids and phospholipids [3], thus contributing to the cellular antioxidant defense system.

The cytotoxic effect of the antitumor drug CDDP (cisplatin, cis-diamine dichloroplatinum) is determined by its DNA-alkylating effect, suppression of transcription, translation, and reparation of DNA, activation of apoptosis and/or necrosis [14]. However, there are a number of reports on the relationship between antitumor effect of CDDP with generation of reactive oxygen species [6,9], which is largely determined by reduced intracellular content of glutathione, an important component of cellular antioxidant defense system, activation of MAP kinases, mitochondrial dysfunction, and activation of NADPH-oxidase.

These findings attest to an important role of Prx1, Prx2, Prx3, and Prx6 in redox-dependent mechanisms of the resistance of the studied cells to CDDP. Taking into account the fact that Prx can regulate cell signaling via modulation of H_2O_2 level [15], we can discuss their role in the transduction of redox-dependent signal during the formation of CDDP resistance of tumor cells.

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